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Studies on the efficacy of Chloramine T trihydrate (*N*-chloro-*p*-toluene sulfonamide) against planktonic and sessile populations of different *Legionella pneumophila* strains

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Abstract

Effectiveness of Chloramine T trihydrate (*N*-chloro-*p*-toluene sulfonamide) on both planktonic and sessile populations of different *Legionella pneumophila* strains was assessed. Although Chloramine T is a recommended commercial formulation for disinfecting cooling towers, there is a lack of published data about the efficacy of this compound against both planktonic and sessile populations of *L. pneumophila.* Planktonic *L. pneumophila* strains were suspended in tap water and sessile *L. pneumophila* strains were grown on stainless steel which is used in the construction of cooling towers, followed by exposure to the biocide. The sensitivity of both planktonic and sessile populations of *L. pneumophila* strains was different. The biocide was found effective below recommended dosages (0.1–0.3%) against planktonic populations of *L. pneumophila*, whereas it was determined that higher dosages than those recommended were required for sessile populations of *L. pneumophila*. The results indicated that studying only the planktonic populations of *L. pneumophila* for biocide tests might not be sufficient to provide information about the optimum dosage and contact time. Therefore, efficacy has to be tested on both planktonic and sessile bacteria.

Keywords: Biocides; Biofilm; Chloramine T trihydrate; *Legionella pneumophila*; Biocidal activity; Disinfection

Introduction

Legionellae are widespread in the environment; they are generally present in very low or undetectable concentrations in natural aquatic environments (Murga et al., 2001; Walker et al., 1999; Winn, 1995). These bacteria may be introduced via the municipal water supply (States et al., 1987; Tison and Seidler, 1983; Zeybek et al., 2003) into man-made water systems that can serve as *Legionella* amplifiers and disseminators (Elsmore, 1986; Green and Pirrie, 1993; Soracco et al., 1983).

Legionellosis is most often acquired through exposure to contaminated water either by inhalation of aerosols or by aspiration following ingestion (Struelens et al., 1993). The infective dose for humans can be assumed to be low – possibly even a single cell – (Addis, 1989; Botzenhart, 2000; Elsmore, 1986) since *Legionella* infections have frequently been traced to contaminated aerosols generated at distances over 6 km and are a potential risk around buildings, such as hospitals and hotels (Tran Minh et al., 2005). In addition to

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aerosol forms, in building water systems, legionellae, like most other aquatic bacteria often exist as attached complex consortia, i.e. biofilms, on plumbing fixtures and heating and ventilating equipment (Green and Pirrie, 1993; Murga et al., 2001). Biofilms and sediments in plumbing systems are potential niches for the growth of *Legionella* (Kooij and Veenendaal, 2002) and can cause problems such as fouling (Johansen et al., 1997), increased resistance to antimicrobial compounds (Green and Pirrie, 1993; Kool, 2002; Surman et al., 1993; Viera et al., 1999), decreased heat transfer from heat exchangers (Wright et al., 1991), and corrosion of metallic substrata (Momba and Binda, 2002).

Because of the potential for any water system to harbor, amplify and disseminate legionellae, control measures need to be considered for all water systems. Therefore, in order to prevent legionellosis outbreaks, contaminated water systems must be disinfected and the most effective and practical biocides need to be evaluated (Elsmore, 1986). Various biocides have been examined for their efficacy in both laboratory simulations and field treatment of *Legionella* contaminations on cooling towers and in potable water systems (Donlan et al., 2002; Elsmore, 1986; Gao et al., 2000; Green and Pirrie, 1993; Kool, 2002; Skaliy et al., 1980; Surman et al., 1993; Swango et al., 1987; Viera et al., 1999; Walker et al., 1999). In this study, we investigated Chloramine T trihydrate (*N*-chloro-*p*-toluene sulfonamide) which is a commercial formulation recommended by manufacturers for inhibiting biological growth in cooling towers. Chloramine T [$C_7H_7SO_2NNaCl$ ($3H_2O$)] should be considered different from monochloramine. Pure monochloramine (NH_2Cl) is a colorless, unstable liquid and it is produced by adding chlorine to a solution containing ammonia, whereas Chloramine T is an organic compound produced by chlorinating benzene sulfonamide or *para*-toluene sulfonamide (Tibbetts, 1995). Chloramine T is a potential alternative to chlorine, safe for humans, non-cytotoxic, stable in solution even at elevated temperatures, has no risk of inducing bacterial resistance, and is readily biodegradable, thus, environment-friendly. Chloramine T has no or only a minor corrosive effect on common industrial materials such as stainless steel, aluminum, and various polymers (Axcentive, undated). Chloramine-T is capable of binding to enzymes and alters their characteristics (Haneke, 2002).

Although Chloramine T is recommended for disinfecting cooling towers, there is a lack of published data about the efficacy of this compound against both planktonic and sessile populations of legionellae. Therefore, in the current work inhibitory characteristics of this biocide was investigated against both suspension and biofilm bacteria.

Materials and methods

Test organisms

All tests were performed with three different *Legionella pneumophila* serogroup 1 strains which were confirmed by monoclonal antibody tests (Oxoid, *Legionella* Latex Test Kit). C1 and C2 strains were isolated from a cooling tower and potable water in different buildings in the vicinity of Istanbul, and a standard strain (SG 1 ATCC 33152) was obtained from Hertfordshire University Biodeterioration Center. Since repeated subculturing on artificial media may affect the resistance of the organisms to biocides and may also cause mutations, strains were maintained individually in private phosphate buffer and glycerin suspension and stored at −70 °C in cryotubes; freeze-dried cultures were not subcultured more than 3 times (Elsmore, 1993; Skaliy et al., 1980; Soracco et al., 1983). For experimental use, freeze-dried cultures of the strains were grown on buffered charcoal yeast extract agar (BCYEA, Oxoid) at 37 °C in an atmosphere of 2.5% CO_2. After cultures reached late log phase, cells were harvested and a suspension was prepared turbidimetrically to a concentration of McFarland 1 standard in Cl_2-free sterile tap water. The suspension was diluted in 1/10 ratio and used in the experiments.

Biocide

Several dosages of Chloramine T trihydrate (15,000, 10,000, 5000, 2000, 1000, 100, 10, 1 mg/l), were prepared in sterile demineralized water. Concentrations of 5000, 2000, 1000, 100, 10, 1 mg/l were used for suspension bacteria and of 15,000 and 10,000 mg/l were used for attached bacteria. Free chlorine doses were measured by the N, *N*-diethyl-*p*-phenylene diamine (DPD) procedure and ranged from 5 to 0.25 mg/l. Dosages for suspended bacteria were determined by minimum inhibitory concentration (MIC) tests.

Preparation of coupons

Stainless steel coupons (1 cm^2, 0.8 mm in thickness) were cleaned using a neutral detergent (Triton) and then rinsed with water before autoclaving (120 °C, 20 min). After sterilization and testing for the presence of any residual detergent, the coupons were placed onto bacterial lawns on BCYEA plates using sterile forceps and the plates were incubated at 37 °C for 7 days. Plates were then examined for the presence of growth inhibition zones around the coupons (Johansen et al., 1997).